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# *In utero* exposure to organochlorine pesticides and early menarche in the Avon Longitudinal Study of Parents and Children

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## Abstract

**Introduction—**Epidemiologic data supporting the role of organochlorine pesticides in pubertal development are limited.

**Methods—**Using a nested case-control design, serum collected during pregnancy from mothers of 218 girls who reported menarche before 11.5 years of age (cases) and 230 girls who reported menarche at or after 11.5 years of age (controls) was analyzed for 9 organochlorines and metabolites. We analyzed the association between *in utero* organochlorine concentrations and early menarche using multivariate logistic regression controlling for mother's age at menarche, or mother's prenatal BMI.

**Results—**We did not observe an association between *in utero* exposure to HCB, β-HCH, ϒ-HCH, p,p′-DDT, p,p′-DDE, oxychlordane or trans-nonachlor and early menarche.

**Conclusions—**This study is the first to examine the association between *in utero* exposure to HCB, β-HCH, ϒ-HCH, oxychlordane or trans-nonachlor and early menarche. *In utero* exposure to organochlorine pesticides does not appear to have a role in the timing of menarche in this study.

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**Keywords**

ALSPAC; Endocrine disrupting compounds; Organochlorine pesticides; Puberty; Menarche

## 1. Introduction

Early menarche, a marker for early puberty, is a risk factor for adverse adult health outcomes including pre- and post-menopausal breast cancer, ovarian cancer, and polycystic ovarian syndrome (Gail et al., 1989; Golub et al., 2008; Moorman et al., 2009; Warner et al., 2013). Previous studies have observed a secular trend towards early puberty in American and European girls (Euling et al., 2008; McDowell et al., 2007; Semiz et al., 2008; Aksglaede et al., 2008; Aksglaede et al., 2009a). There is evidence to suggest a decrease in age at menarche (McDowell et al., 2007; Semiz et al., 2008) and a decrease in age at breast development (Semiz et al., 2008; Aksglaede et al., 2009a). Variability in the onset of puberty has been associated with several genetic and non-genetic factors (Parent et al., 2003). Studies suggest that the declining trend in age at onset of puberty cannot be explained by genetics and obesity alone, and that other environmental factors, such as exposures to endocrine disrupting compounds (EDCs) are involved (Parent et al., 2003; Mouritsen et al., 2010).

Several organochlorine pesticides have been banned or restricted in most countries for decades because of their persistence in the environment and their association with adverse effects in humans and wildlife (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), 2009). However, they are still in use especially in developing countries, mainly to control vector-borne diseases (Bergman et al., 2013). Human exposure to organochlorine pesticides occurs primarily through diet, particularly consumption of fatty foods such as meat, fish, and dairy products (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), 2009). Organochlorine pesticides can be transferred to the developing fetus through the placenta and to newborns through breast milk. (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), 2009) Animal studies suggest that some organochlorine pesticides can act as EDCs by exhibiting weak estrogenic properties associated with early onset of puberty (Clement and Okey, 1972; Gellert et al., 1972). Mechanisms of organochlorine pesticides endocrine activity reported in literature include binding with hormone receptors (Mrema et al., 2013; Raiser et al., 2006), altering hormonal pathways by directly inhibiting enzyme activities responsible for the synthesis of precursors of hormones (Mrema et al., 2013), and stimulating the secretion of the gonadotrophin releasing hormone (GnRH) (Raiser et al., 2006). Exposure to organochlorine pesticides can therefore induce early puberty and eventual early menarche attainment through activation of the hypothalamic GnRH pulse generator or estrogen receptor independent of hypothalamic-pituitary axis (Raiser et al., 2006). Additionally, hormone actions during fetal development can be more potent than hormone actions in adults, and the effects permanent (Bergman et al., 2013). It has been suggested that the *in utero* environment, critical for fetal development, may affect health outcomes later in life such as onset of menarche (Adair, 2001; Kaprio et al., 1995), and that *in utero* exposure to the estrogenic effect of organochlorine pesticides

can result in *in utero* programming of the age at menarche resulting in early menarche (Vasiliu et al., 2004).

Epidemiologic data supporting the role of organochlorine pesticides in pubertal development are limited (Bergman et al., 2013). Reasons include limited capacity of some studies to show temporal relation between exposure and puberty onset, limited studies on the role of background exposures, limited ability to assess confounding, and limited number of organochlorines studied (Crain et al., 2008; Bergman et al., 2013; Buck Louis et al., 2008). Data on the role of *in utero* exposures, including organochlorine pesticides, in puberty onset are also limited (Crain et al., 2008; Buck Louis et al., 2008). Using a nested case-control study design, we measured gestational maternal serum concentrations of organochlorine pesticides, a proxy for *in utero* exposure to organochlorine pesticides, and examined the association with early onset of menarche, as a marker for early puberty, measured prospectively in the population-based Avon Longitudinal Study of Parents and Children (ALSPAC) in Bristol, United Kingdom (U.K.).

## 2. Study design and methods

### 2.1. Study population

Information about ALSPAC and participant recruitment has been described elsewhere (Fraser et al., 2013; Boyd et al., 2013). Pregnant women residing in Bristol and the surrounding area, in the South west of England, were eligible to participate if their expected delivery date was between and including the dates 1st April, 1991 and 31st December, 1992. The total sample size was 15,247 pregnancies resulting in 14,775 live births. A fully searchable data dictionary is available at http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/.

The selection of cases and controls for the nested case-control study has already been described in detail (Christensen et al., 2011). Briefly, from the cohort of 14,775 live births, 3682 singleton girls returned at least 2 ‘Growing and Changing’ puberty questionnaires between the ages of 8 to 13 years, which recorded self-reported age at onset of menarche and month and year of first period. This questionnaire was sent to the study participants every year from ages 8 to 17 (except at age 12). The puberty questionnaire is available at http://www.bristol.ac.uk/media-library/sites/alspac/migrated/documents/ques-cb16a-mum-and-daughter-at-8.pdf. Girls were ranked according to age at menarche, and 11.5 years was selected as the cut-off for earlier age at menarche to satisfy sample size and power needed for the case-control study. The median age at onset of menarche (95% confidence interval) for the larger cohort of girls (n = 3938) that returned at least one puberty questionnaire was reported as 12.87 years (10.82–12.91) (Christensen et al., 2010). Of the 3682 girls who returned two or more questionnaires, 218 had earlier menarche, defined as first menstruation before 11.5 years (cases) and also had a maternal serum sample that could be analyzed. A random sample of 230 controls who had menarche at 11.5 years or older and had a maternal serum sample that could be analyzed, was selected from the same group of 3682 singleton girls. Serum samples were collected from the mothers during routine antenatal care at random times during pregnancy (1991–1992) (Fraser et al., 2013; Boyd et al., 2013). The serum samples were banked at the University of Bristol at +4 °C for 0–4 days after initial

collection (or frozen at −20 °C for 0–6 days after initial collection), then aliquoted and refrozen at −20 °C until analysis. Human subject protection was assessed and approved by the ALSPAC Law and Ethics Committee, the Local Research Ethics Committees, and the Centers for Disease Control and Prevention (CDC) Institutional Review Board (Christensen et al., 2011).

### 2.2. Exposure assessment

Maternal serum samples for this study were sent to the National Center for Environmental Health (NCEH), CDC in 2008 for analysis. The NCEH laboratory measured the following 9 organochlorine pesticide analytes by gas chromatography isotope dilution high resolution mass spectrometry (Sjodin et al., 2004): Hexachlorobenzene (HCB), isomers of hexachlorocyclohexane (*i.e.*, β-hexachlorocyclo-hexane (β-HCH) and ϒ-hexachlorocyclohexane (ϒ-HCH/Lindane)), chlorodane-related pesticides (*i.e.*, oxychlorodane (oxychlor) and trans-nonachlor (T-nona)), Mirex, and isomers of DDT (*i.e.*, 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (p,p′-DDE); 2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethane (o,p′-DDT); and 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (p,p′-DDT)). Concentrations were reported on a whole weight basis (pg/g serum) and lipid-adjusted basis (ng/g lipid) corrected for total serum lipid levels. The total serum lipid levels were calculated from the measured concentration of total cholesterol and triglycerides using the equation 'Total lipid (g/L) = 2.27 × Total Cholesterol (g/L) + Total triglycerides + 0.623′ 0.623′ as reported in Phillips et al. (1989) The limit of detection (LOD) was defined as the highest of (i) three times the standard deviation of blanks analyzed in parallel with the unknowns and (ii) the lowest calibration point having a signal to noise ratio greater than three (Sjodin et al., 2004).

Some of the organochlorine values reported were below the LOD, therefore, in order to calculate the median, and 25th and 75th percentiles, we used survival analysis methods (proc Lifetest for non-parametric estimation) with SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA) to account for left censored values, *i.e.*, <LOD (Gillespie et al., 2010). In order to use methods intended for right-censored data on left-censored data, we transformed the data by subtracting a constant from the pesticide concentrations, and after calculations, transformed the data back to the original units. We calculated the median, and 25th and 75th percentiles for lipid-adjusted values for organochlorines detected ≥LOD in >50% of the study participants. For organochlorines detected in <50% of the study participants we only calculated the percent >LOD.

### 2.3. Association between early menarche and organochlorine serum concentrations

To assess the association between early menarche and organochlorine serum concentrations, we substituted values <LOD with $\mathrm{LOD}/\sqrt{2}$ and natural log transformed the values before analysis for organochlorines measured in >50% of study participants. Serum sample weights for this study ranged from 0.12 g to 1.23 g, and smaller samples (<0.4 g) tended to have higher LODs (*i.e.*, less ability to detect low levels of organochlorine concentrations) (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), 2009). These higher LODs could bias analyses when substituting values <LOD with

$\mathrm{LOD}/\sqrt{2}$; therefore, we plotted the lipid-adjusted LOD against the sample weight (see supplement), selected an LOD cut-off of 13 ng/g lipid, and excluded participants with a lipid-adjusted LOD ≥ 13 ng/g lipid (Sjodin et al., 2014). Using this criteria, we removed 10 study participants from the analysis of p,p′-DDT, which was the only organochlorine evaluated that had values <LOD with LOD ≥13 ng/g lipid.

We also dichotomized exposure to these organochlorines as ≥median or <median using the distribution of the controls. For organochlorines measured in <50% of study participants we dichotomized exposure as "yes" for values measured ≥LOD and "No" for values <LOD.

We identified *a priori*, the following 10 covariates that could be associated with the exposure or outcome, and assessed them for potential confounding: mother's race (White or non-White) (McDowell et al., 2007; Biro et al., 2010; Rubin et al., 2009), mother's pre-natal body mass index (BMI) (<18.5 (underweight), 18.5–24.9 (normal), 25–29.9 (over-weight), or ≥30 (obese)) (Rubin et al., 2009), mother's age at menarche (8–11, 12–14, or ≥15 years) (Rubin et al., 2009), mother's age at delivery (<20, 20–24, 25–29, 30–39, or ≥40 years) (Schade and Heinzow, 1998), socio-economic status (using mother's education as a proxy) (certificate of secondary education (CSE)/none, vocational, O-level, A-level, or degree) (Parent et al., 2003), child's birth order (first born, second born, or third born or later) (Rubin et al., 2009; Schade and Heinzow, 1998), child's BMI at age 7 ($Kg/m^2$) (Biro et al., 2010; Aksglaede et al., 2009b), duration of breast feeding (never, <6, or ≥6 months) (Schade and Heinzow, 1998), child's birth weight (<2500 or ≥2500 g) (Ruder et al., 2010), and gestation trimester when serum sample was collected (≤12, 13–28, or ≥29 weeks) (Longnecker et al., 1999).

We used logistic regression to analyze the association between early menarche and organochlorine pesticide exposure. We included potential confounders with p-value (p) <0.25 (Mickey and Greenland, 1989) in the multivariate logistic model.

## 3. Results

Compared to the controls, cases were more likely to have a mother who had onset of menarche between the ages of 8 and 11 years, or who was overweight or obese before pregnancy (Table 1). Cases were also more likely to be a first born child or have a mother who was of non-white race, compared to the controls. Cases had significantly higher pre-pubertal BMIs at age 7 compared to controls ($p < 0.0001$).

HCB, β-HCH, p,p′-DDE, and p,p′-DDT were detected above the LOD in >50% of the study participants while T-nona, oxychlor, ϒ-HCH, o, p′-DDT and mirex were detected above the LOD in <50% of the study participants (Table 2). HCB was the most frequently detected organochlorine (100%) followed by p,p′-DDE (99.8%), β-HCH (98.1%), p,p′-DDT (92.5%), T-nona (31.2%), oxychlor (26.9%), ϒ-HCH (20.8%), o, p′-DDT (1.4%) and mirex (0.5%). Because of low detection >LOD, o,p′-DDT and mirex were not included in further analyses. There were no significant differences in exposure to any of the organochlorine pesticide concentrations between cases and controls (Table 2). We used lipid-adjusted

organochlorine concentrations in all our analyses (See supplement for tables using whole weight concentrations).

We did not find an association between *in utero* exposure to organochlorine pesticides and early menarche (Tables 3, 4 and 5). We examined the organochlorine pesticide and early menarche association using 3 lipid-adjusted variables for HCB, β-HCH, p,p′-DDE, and p,p′-DDT: log transformed continuous, dichotomized (<median or ≥median), and categorized into quartiles. We dichotomized lipid-adjusted T-nona, oxychlor, and ϒ-HCH concentrations as <LOD or ≥LOD. In the unadjusted models, the odds of early menarche for each unit increase of logged organochlorine pesticide concentration (odds ratio (OR)) and 95% confidence interval (CI) were OR = 1.06 (95% CI: 0.67, 1.68) for HCB, OR = 0.91 (95% CI: 0.64, 1.29) for β-HCH, OR = 0.91 (95% CI: 0.70, 1.19) for p,p′-DDE and OR = 1.06 (95% CI: 0.72, 1.54) for p,p′-DDT (Table 3). In the adjusted multivariate models, all odds ratios were <1; OR = 0.93 (95% CI: 0.58–1.50) for HCB, OR = 0.85 (95% CI: 0.59–1.22) for β-HCH, and OR = 0.97 (95% CI: 0.65–1.42) for p,p′-DDT. None of the unadjusted or adjusted associations were significant (95% CI included the null value, 1, and p value > 0.05). The results were similar for HCB and β-HCH exposure dichotomized as ≥median or <median (Table 4). However, the direction of association changed for DDE (unadjusted OR = 1.23 (95% CI: 0.80–1.85)) and DDT (adjusted OR = 1.13 (95% CI: 0.74–1.73)). None of the associations were significant. As a sensitivity analysis, we categorized exposure to these organochlorines in quartiles (<25th, ≥25th–<50th, ≥50th–<75th, or ≥75th percentiles). The odds of early menarche for *in utero* exposure to ≥75th percentile compared to <25th percentile (reference) remained similar to those presented in Table 3, except the unadjusted odds for p,p′-DDT which was <1 (Table 5). For exposure to oxychlor, T-nona and ϒ-HCH dichotomized as ≥LOD or <LOD, the unadjusted and adjusted odds ratios were <1 except for T-nona with unadjusted odds ratio = 1.05 (95% CI: 0.69–1.62) (Table 4). We did not examine the association between early menarche and o,p′-DDT or mirex because very few participants had detected values >LOD (1.4% and 0.5% respectively).

We also performed a sensitivity analysis using different confounders. We selected *a priori* 4 covariates typically used in other puberty studies: mother's age at menarche, mother's age at delivery, mother's education and duration of breastfeeding. For the early menarche-logged organochlorine pesticide exposure association analysis, the results were similar to those presented in Table 3, but the direction of adjusted odds of p,p′-DDT changed to >1 (data not shown). For dichotomous exposure, the results were similar to those presented in Table 4 (data not shown). None of the associations were significant.

We also explored the organochlorine and early menarche association, only in the subset of controls, using linear regression analysis, and did not find any association (see supplement).

## 4. Discussion

### 4.1. Puberty

This study is the first to analyze the association between *in utero* exposure to HCB, β-HCH, ϒ-HCH, oxychlordane or T-nonachlor and early menarche in girls. Two previous studies have analyzed the association between *in utero* exposure to DDE and pubertal development

(Vasiliu et al., 2004; Gladen et al., 2000). Similar to the Gladen et al. study in girls born between 1978 and 1982 (Gladen et al., 2000), our study did not find an association between *in utero* exposure to DDE, or other organochlorine pesticides, and age at menarche. The lipid-adjusted median DDE concentration for the Gladen et al. (2000) study population was 2400 ppb, while the lipid-adjusted median DDE concentration for our study population was 311 ng/g (ppb). In comparison, a study by Vasiliu et al. (2004) among children born between 1950 and 1980 found that an increase of 15 μg/l of DDE *in utero* reduced age at menarche by one year; however, this association was no longer significant after controlling for BMI at age of menarche. The study authors hypothesized that the estrogenic effect of DDE *in utero* could result in earlier age at menarche as supported by findings in other studies that reported lower age at menarche in female twins compared to girls exposed to male co-twins *in utero* (Kaprio et al., 1995; Vasiliu et al., 2004). The population in the Vasiliu et al. study may have been exposed to potentially higher amounts of DDE due to high maternal consumption of contaminated fish in the Great Lakes. The median *in utero* DDE concentration was 7 μg/l for girls who attained menarche between the ages of 9 and 11 years, 4.2 μg/l and 3.8 μg/l for girls who attained menarche between the ages 12–14 years and 14–17 years respectively. The study did not report lipid-adjusted concentrations. This previous study was smaller (N = 151), with only 22 girls with age at onset of menarche from 9–11 years (Vasiliu et al., 2004). In this present study, we were also able to assess additional characteristics of the mother and child for confounding potential, *e.g.*, child's pre-pubertal BMI, collected prospectively through puberty.

Our study analyzed the association between *in utero* exposure to organochlorine pesticides and age at menarche, but not onset of puberty which is typically represented by a Tanner stage 2 of breast development. Even though *in utero* exposure to organochlorine pesticides does not appear to impact menarche timing in our study, we should not rule out the possibility of an impact on age at puberty onset. The fact that the timing of onset of puberty in girls seems to be decreasing (Euling et al., 2008; Aksglaede et al., 2009a) while the timing of menarche recently appears to be more stable (Juul et al., 2006) lends support to the exploration of such associations.

### 4.2. Exposure

U.K. population exposure studies with serum samples collected from 2001–2003 showed >50% lower levels of p,p′-DDT, p,p′-DDE, HCB and β-HCH median concentrations compared to our study (Kalantzi et al., 2004; Thomas et al., 2006). This decreasing trend has also been observed in other developed countries such as the United States where these pesticides are no longer in use or have been restricted (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), 2009). DDT was banned in the United Kingdom in 1986 (Rogan and Chen, 2005). Twelve persistent organic pollutants including DDT, HCB and chlordane were banned or restricted globally in a 2004 treaty at the Stockholm Convention on Persistent Organic Pollutants, and HCH was one of the 9 pollutants added to the list in a 2009 amendment (The Stockholm Convention, 2008). However, some organochlorines, such as DDT, are still in use especially in developing countries, mainly for vector-borne disease control (Bergman et al., 2013).

### 4.3. Study limitations

This study may be subject to selection bias due to differences in characteristics of participants who did not respond to the puberty questionnaire and therefore were excluded from this study (Christensen et al., 2011). The removal of some records from the analysis of p,p′-DDT could bias the study results; however, when we included all the samples of p,p′-DDT in the analyses, the results were similar. We excluded from our analyses records with missing covariate data and lipid-adjusted values of the organochlorine concentrations; this exclusion could also bias our results. However, when we imputed values for missing data using SAS 9.3 and re-ran the analyses, the results remained similar to those presented in Tables 3 and 4. The study may be underpowered to detect associations between gestational organochlorine pesticide concentrations and early menarche due to a small sample size.

## 5. Conclusion

This is the first study to examine the association between *in utero* exposure to HCB, β-HCH, ϒ-HCH, oxychlordane or trans-nonachlor and early menarche. *In utero* exposure to organochlorine pesticides appears to have no role in the timing of menarche in this study. Since populations are often exposed to combinations of pesticides and other chemicals in the environment, future work will include examining the effects of multiple EDC exposures, including organochlorine pesticides, on onset of puberty in the ALSPAC cohort.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2016.06.001.

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**Table 1**

Study population characteristics.

| Characteristic | Cases (N = 218) | | Controls (N = 230) | | p-Value[a] |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Mother's race | | | | | 0.06 |
| White | 205 | 96.7 | 218 | 99.5 | |
| Other | 7 | 3.3 | 1 | 0.5 | |
| Missing | 6 | | 11 | | |
| Mother's age at delivery (yrs) | | | | | 0.43 |
| <20 | 1 | 0.5 | 7 | 3.1 | |
| 20–24 | 43 | 19.9 | 41 | 17.9 | |
| 25–29 | 83 | 38.4 | 81 | 35.4 | |
| 30–39 | 86 | 39.8 | 96 | 41.9 | |
| >40 | 3 | 1.4 | 4 | 1.8 | |
| Missing | 2 | | 1 | | |
| Mother's education[b] | | | | | 0.88 |
| CSE/none | 31 | 14.8 | 26 | 11.9 | |
| Vocational | 17 | 8.1 | 15 | 6.9 | |
| O-level | 67 | 31.9 | 73 | 33.3 | |
| A-level | 61 | 29.1 | 66 | 30.1 | |
| Degree | 34 | 16.2 | 39 | 17.8 | |
| Missing | 8 | | 11 | | |
| Mother's prenatal BMI | | | | | 0.01 |
| <18.5 | 7 | 3.6 | 11 | 5.4 | |
| 18.5–24.9 | 129 | 65.5 | 161 | 78.5 | |
| 25–29.9 | 42 | 21.3 | 21 | 10.2 | |
| >30 | 19 | 9.6 | 12 | 5.9 | |
| Missing | 21 | | 25 | | |
| Mother's age at menarche (yrs) | | | | | 0.0004 |
| 8–11 | 63 | 32.5 | 30 | 15.2 | |
| 12–14 | 119 | 61.3 | 153 | 77.3 | |

| Characteristic | Cases (N = 218) | | Controls (N = 230) | | p-Value[a] |
|---|---|---|---|---|---|
| | N | % | N | % | |
| ≥15 | 12 | 6.2 | 15 | 7.6 | |
| Missing | 24 | | 32 | | |
| Child's birth order | | | | | 0.07 |
| First born | 110 | 53.9 | 98 | 45.6 | |
| Second born | 57 | 27.9 | 83 | 38.6 | |
| Third born or later | 37 | 18.1 | 34 | 15.8 | |
| Missing | 14 | | 15 | | |
| | Median | Quartile | Median | Quartile | |
| Child's BMI at age 7 | 17.2 | 15.9–18.9 | 15.8 | 14.9–16.9 | <0.0001 |
| Child's age at menarche (yrs) | 11 | 10.8–11.3 | 12.75 | 12.2–13.3 | |

[a] Comparison between cases and controls using logistic regression.

[b] CSE = certificate of secondary education, O-level = ordinary level, A-level = advanced level.

**Table 2**

Lipid-adjusted gestational serum concentrations (ng/g lipid) by case/control status and overall.

| Organochlorine | Total | | Cases | | Controls | | p-Value[c] |
|---|---|---|---|---|---|---|---|
| | N (% > LOD[a]) | Median (IQR)[b] | N (% > LOD) | Median (IQR) | N (% > LOD) | Median (IQR) | |
| HCB | 372 (100) | 50.2 (37.9–63.6) | 184 (100) | 50.9 (38.3–64.7) | 188 (100) | 49.8 (37.8–63.5) | 0.64 |
| β-HCH | 374 (98.1) | 47.2 (34.7–62.6) | 186 (98.4) | 47.4 (34.3–59.6) | 188 (97.9) | 47.2 (35.6–63.7) | 0.39 |
| p,p′-DDE | 428 (99.8) | 311.0 (192.5–499.0) | 210 (99.5) | 314.0 (184.0–522.0) | 218 (100) | 309.5(200.0–484.0) | 0.63 |
| p,p′-DDT | 362 (92.5) | 11.0 (8.1–16.5) | 183 (92.3) | 11.5 (7.9–16.7) | 179 (92.7) | 10.1 (8.1–16.5) | 0.80 |
| Lipids (md/dL) | 429 | 576.4 (504.0–697.1) | 210 | 571.9 (500.6–681.1) | 219 | 582 (506.7–710.0) | |

[a] Each biological sample had an individual limit of detection based on the sample's weight and lipid concentration.

[b] Median and IQR values were derived using Proc Lifetest for non-parametric estimation of values <LOD.

[c] Comparison between cases and controls using Wilcoxon Rank Sum Test.

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## Table 3

Association between lipid-adjusted organochlorine gestational serum concentrations (ng/g lipid) and early menarche (logged organochlorine concentration).

| Organochlorine | Unadjusted OR (95% CI)[a, e] | Adjusted OR (95% CI)[a, e] | Adjusted p value |
|---|---|---|---|
| HCB | 1.06 (0.67–1.68) | 0.93 (0.58–1.50)[b] | 0.77 |
| β-HCH | 0.91 (0.64–1.29) | 0.85 (0.59–1.22)[b] | 0.37 |
| p,p′-DDE | 0.91 (0.70–1.19) | | 0.50[c] |
| p,p′-DDT | 1.06 (0.72–1.54) | 0.97 (0.65–1.42)[d] | 0.84 |

[a]Odds of early menarche for a unit increase of logged organochlorine pesticide concentration.

[b]Adjusted for mother's age at menarche.

[c]Unadjusted p value. No potential confounders identified.

[d]Adjusted for mother's prenatal BMI.

[e]Number of cases, number of controls used in both unadjusted and adjusted models: HCB (184, 188); β-HCH (186, 188); PP′-DDE (210, 218); PP′-DDT (183, 179).

**Table 4**

Association between lipid-adjusted organochlorine gestational serum concentrations (ng/g lipid) and early menarche (dichotomous organochlorine concentration).

| Organochlorine | Unadjusted OR (95% CI)[f] | Adjusted OR (95% CI)[f] | Adjusted p value |
|---|---|---|---|
| HCB | 1.09 (0.73–1.64)[a] | 0.98 (0.65–1.49)[a, c] | 0.93 |
| β-HCH | 0.98 (0.65–1.47)[a] | 0.92 (0.61–1.39)[a, c] | 0.69 |
| p,p′-DDE | 1.02 (0.70–1.49)[a] | | 0.92[d] |
| p,p′-DDT | 1.22 (0.80–1.85)[a] | 1.13 (0.74–1.73)[a, e] | 0.57 |
| Oxychlor | 0.73 (0.46–1.14)[b] | 0.67 (0.43–1.07)[b, c] | 0.09 |
| T-nona | 1.05 (0.69–1.62)[b] | 0.99 (0.64–1.55)[b, e] | 0.99 |
| ϒ-HCH | 0.83 (0.53–1.32)[b] | | 0.43[d] |

[a] Odds of early menarche for *in utero* exposure ≥ median compared to <median using distribution of controls.

[b] Odds of early menarche for *in utero* exposure ≥ LOD compared to <LOD.

[c] Adjusted for mother's age at menarche.

[d] Unadjusted p value. No potential confounders identified.

[e] Adjusted for mother's prenatal BMI.

[f] Number of cases, number of controls used in both unadjusted and adjusted models: HCB (184, 188); β-HCH (186, 188); PP′-DDE (210, 218); PP′-DDT (183, 179); oxychlordane (194, 197); trans-nonachlor (189, 196); ϒ-HCH (218, 229).

## Table 5

Association between lipid-adjusted organochlorine gestational serum concentrations (ng/g lipid) and early menarche (organochlorine concentration categorized in quartiles; <25th, ≥25th–<50th, ≥50th–<75th, and ≥75th percentiles).

| Organochlorine | Unadjusted OR (95% CI)[a, e] | Adjusted OR (95% CI)[a, e] | Adjusted p value |
|---|---|---|---|
| HCB | | | |
| <25th | 1 (reference) | 1 (reference) | |
| ≥25th–<50th | 0.92 (0.51–1.64) | 0.98 (0.54–1.77)[b] | 0.99 |
| <50th–≥50th | 1.07 (0.60–1.89) | 1.06 (0.59–1.90)[b] | 0.67 |
| ≥75th | 1.02 (0.57–1.82) | 0.89 (0.49–1.60)[b] | 0.59 |
| β-HCH | | | |
| <25th | 1 (reference) | 1 (reference) | |
| ≥25th–<50th | 0.71 (0.39–1.26) | 0.73 (0.40–1.32)[b] | 0.53 |
| <50th–≥50th | 0.91 (0.52–1.58) | 0.89 (0.51–1.56)[b] | 0.67 |
| ≥75th | 0.76 (0.43–1.35) | 0.71 (0.40–1.27)[b] | 0.42 |
| p,p′-DDE | | | |
| <25th | 1 (reference) | | |
| ≥25th–<50th | 0.64 (0.37–1.10) | | 0.17[c] |
| <50th–≥50th | 0.73 (0.43–1.25) | | 0.53[c] |
| ≥75th | 0.93 (0.56–1.57) | | 0.40[c] |
| p,p′–DDT | | | |
| <25th | 1 (reference) | 1 (reference) | |
| ≥25th–<50th | 0.56 (0.29–1.06) | 0.53 (0.28–1.02)[d] | 0.06 |
| <50th–≥50th | 0.99 (0.57–1.74) | 0.91 (0.52–1.60)[d] | 0.41 |
| ≥75th | 0.89 (0.50–1.60) | 0.81 (0.45–1.47)[d] | 0.90 |

[a] Odds of early menarche for quartiles ≥25th–<50th, ≥ 50th–<75th, or ≥75th compared to <25th.

[b] Adjusted for mother's age at menarche.

[c] Unadjusted p value. No potential confounders identified.

[d] Adjusted for mother's prenatal BMI.

[e] Number of cases, number of controls used in both unadjusted and adjusted models: HCB (184, 188); β-HCH (186, 188); PP′-DDE (210, 218); PP′-DDT (183, 179).